

AMENDMENTS

Amendments to the Claims

1. (Previously presented) A method of identifying a compound that either reduces or increases a biological persistence of a BoNT/A, the method comprising a test localization assay comprising the steps of:

- (a) contacting a cell that comprises a BoNT/A light chain with a test compound, wherein the BoNT/A light chain displays an intracellular localization pattern at the plasma membrane;
- (b) observing the membrane localization pattern of the BoNT/A light chain following contact of the cell with the test compound; and
- (c) comparing the observed BoNT/A light chain membrane localization pattern to a membrane localization pattern of a BoNT/A light chain in a cell in an absence of the test compound;

wherein a reduced membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that reduces the biological persistence of a BoNT/A; and

wherein an increased membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that increases the biological persistence of a BoNT/A.

2. (Cancelled)

3. (Previously presented) The method of claim 1 wherein in step (c) an observed increased biological persistence is about 20% to about 300% more BoNT/A light chain localized to the plasma membrane over time in the cell contacted with the test compound as compared to the BoNT/A light chain localized to the plasma membrane over time in the cell in the absence of the test compound, said more membrane localization pattern being indicative of a test compound that increases the biological persistence of a BoNT/A.
4. (Previously presented) The method of claim 1 wherein in step (c) an observed reduced biological persistence is about 10% to about 90% reduction in plasma membrane localization of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization of the BoNT/A light chain over time in the cell in the absence of the test compound, said reduced membrane localization pattern being indicative of a test compound that reduces the biological persistence of a BoNT/A.
5. (Previously presented) The method of claim 1 further comprising a negative control localization assay comprising the steps of:
 - (a) contacting a cell that comprises the BoNT/A light chain with a localization assay negative control compound, wherein the localization assay negative control compound is a compound known to have no effect on the membrane localization pattern of the BoNT/A light chain in a cell; and
 - (b) determining whether the membrane localization pattern of the BoNT/A light chain in the cell differs following contacting the cell with the localization assay negative control compound compared to the membrane localization pattern of the BoNT/A light chain in the cell in the absence of the localization assay negative control compound,

wherein a change in the membrane localization pattern of the BoNT/A light chain in the cell following contacting the cell with the localization assay negative control compound indicates that the test localization assay results are inconclusive.

6. (Previously presented) The method of claim 1 further comprising a positive control localization assay comprising the steps of:

(a) contacting a cell that comprises the BoNT/A light chain with a localization assay positive control compound, wherein the localization assay positive control compound is a compound known to change the membrane localization pattern of the BoNT/A light chain in a cell; and

(b) determining whether the membrane localization pattern of the BoNT/A light chain in the cell differs following contacting the cell with the localization assay positive control compound compared to the membrane localization pattern of the BoNT/A light chain in the cell in the absence of the localization assay positive control compound,

wherein an absence of change in the localization pattern of the light chain in the cell following contacting the cell with the localization assay positive control compound indicates that the test localization assay results are inconclusive.

7. (Original) The method of claim 1 comprising multiple test localization assays wherein individual test assays are performed using different concentrations of test compound.
8. (Original) The method of claim 1 comprising performing at least a duplicate test localization assays.
9. (Original) The method of claim 1 wherein the cell is selected from the group consisting of: Neuro-2A cells, PC12 cells, SHSY-5Y cells, HIT-T15 cells, HeLa cells, HEK293 cells, and primary and established neuronal culture cells from spinal cord, cortex, hippocampus and dorsal root ganglion.

10. (Previously presented) The method of claim 1 wherein the cell comprises a gene that encodes the BoNT/A light chain, which is expressed to produce the BoNT/A light chain in the cell.
11. (Previously presented) The method of claim 1 wherein a BoNT/A is contacted with the cell in an amount effective to be taken up by the cell, the amount effective to be taken up by the cell being the amount able to produce an identifiable membrane localization pattern of the BoNT/A light chain in the cell.
12. (Previously presented) The method of claim 1 wherein the BoNT/A light chain is labeled.
13. (Previously presented) The method of claim 1 claim 12 wherein the labeled BoNT/A light chain is labeled with a radio-active isotope or a fluorescent marker.
14. (Previously presented) The method of claim 1 wherein the BoNT/A light chain is expressed as a fusion protein comprising a BoNT/A light chain fused with a fluorescent marker.
15. (Previously presented) The method of claim 1 wherein the membrane localization pattern is determined using microscopic techniques that allow for the analysis of changes in subcellular localization, including confocal microscopic systems.
16. (Previously presented) The method of claim 1 further comprising a test enzymatic assay comprising the steps of:
 - (a) contacting a sample containing the BoNT/A light chain with a SNAP-25 substrate in the presence of the test compound; and
 - (b) determining whether the SNAP-25 substrate is processed by the BoNT/A light chain into enzymatic product;

wherein the absence of processing of the SNAP-25 substrate into enzymatic product indicates that the test compound inhibits BoNT/A enzymatic activity, and the enhancement of processing of the SNAP-25 substrate into enzymatic product indicates that the test compound enhances BoNT/A enzymatic activity.

17. (Previously presented) The method of claim 16 further comprising a negative control enzymatic assay comprising the steps of:

- (a) contacting a sample that comprises the BoNT/A light chain with a SNAP-25 substrate in the presence of an enzymatic assay negative control compound or no added compound, wherein the enzymatic assay negative control compound is a compound known not to inhibit BoNT/A enzymatic activity; and
- (b) determining whether the SNAP-25 substrate is processed by the BoNT/A light chain into enzymatic product;

wherein the absence of processing of the SNAP-25 substrate into enzymatic product indicates that test enzymatic assay results are inconclusive.

18. (Previously presented) The method of claim 16 further comprising a positive control enzymatic assay comprising the steps of:

- (a) contacting a sample that comprises the light chain with a SNAP-25 substrate in the presence of an enzymatic assay positive control compound, wherein the enzymatic assay positive control compound is a compound known to inhibit BoNT/A enzymatic activity; and
- (b) determining whether the SNAP-25 substrate is processed by the BoNT/A light chain into enzymatic product;

wherein processing of the SNAP-25 substrate into enzymatic product indicates that test enzymatic assay results are inconclusive.

19. (Original) The method of claim 16 comprising multiple test enzymatic assays wherein individual enzymatic test assays are performed using different concentrations of test compound.
20. (Original) The method of claim 16 comprising performing at least duplicate test enzymatic assays.
21. (Cancelled)
22. (Previously presented) The method of claim 16 wherein the processing of it into enzymatic product is determined by Western blot, ELISA assay, GFP-SNAP assay, FRET assay, or a combination of said assays, using an antibody that specifically binds to uncleaved SNAP-25 substrate and/or enzymatic products.
- 23-44. (Cancelled)
45. (Currently amended) A method of identifying a compound that either reduces or increases a biological persistence of a ~~Clostridial toxin~~ BoNT/A, the method comprising the steps of:
- (a) contacting a cell that comprises a BoNT/A light chain with a test compound, wherein the BoNT/A light chain displays an intracellular localization pattern at the plasma membrane; and
 - (b) determining whether the membrane localization pattern of the BoNT/A light chain is reduced or increased in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain in a cell in the absence of the test compound,
- wherein a reduced membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization

pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that reduces the biological persistence of a BoNT/A; and

wherein an increased membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that increases the biological persistence of a BoNT/A.

46. (Previously presented) The method of claim 45 wherein in step (b) a determined increased biological persistence is about 20% to about 300% more BoNT/A light chain localized to the plasma membrane over time in the cell contacted with the test compound as compared to the BoNT/A light chain localized to the plasma membrane over time in the cell in the absence of the test compound, said more membrane localization pattern being indicative of a test compound that increases the biological persistence of a BoNT/A.

47. (Previously presented) The method of claim 45 wherein in step (b) a determined reduced biological persistence is about 10% to about 90% reduction in plasma membrane localization of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization of the BoNT/A light chain over time in the cell in the absence of the test compound, said reduced membrane localization pattern being indicative of a test compound that reduces the biological persistence of a BoNT/A.

48-55 (Cancelled).

56. (Previously presented) The method of claim 1 wherein in step (c) an observed reduced biological persistence is a more than 20% reduction in BoNT/A light chain density at the plasma membrane over time in the cell contacted with the test compound as compared to the BoNT/A light chain density at the plasma membrane over time in the cell in the

absence of the test compound, said reduced BoNT/A light chain density being indicative of a test compound that decreases the biological persistence of a BoNT/A.

57. (Previously presented) The method of claim 45 wherein in step (b) a determined reduced biological persistence is a more than 20% reduction in BoNT/A light chain density at the plasma membrane over time in the cell contacted with the test compound as compared to the BoNT/A light chain density at the plasma membrane over time in the cell in the absence of the test compound, said reduced BoNT/A light chain density being indicative of a test compound that decreases the biological persistence of a BoNT/A.